CLAIMS

Status of the Claims

Claims 1-31 were originally filed. Claims 14-16 and 21-31 were previously canceled. Claims 3, 5-13, 17, and 20 were previously amended. Herein claims 1 and 18 are presently amended without prejudice or disclaimer of the subject matter therein. Applicant specifically reserves the right to file one or more divisionals on the original subject matter.

In the Claims

1. (Presently Amended) A method for preparing a protein-polymer conjugate comprising:

(a) contacting an insulin protein in an aqueous solution with an amino-reactive derivative of ~~with~~ a hydrophilic polymer in the presence of at least one organic solvent and at least one metal chelator, ~~under conditions that~~ to form ~~promote the formation of~~ a conjugate of the protein and the polymer; and

(b) isolating the conjugate.

2. (Original) The method of claim 1, wherein the insulin protein comprises human insulin.

3. (Previously Amended) The method of claim 1, wherein the hydrophilic polymer is selected from the group consisting of polyethylene glycol, polyethylene glycol/polypropylene glycol copolymers, polyoxyethylated glycerol, and linear, branched and amino-reactive derivatives thereof.

4. (Original) The method of claim 3, wherein the amino-reactive derivative is selected from the group consisting of an aldehyde, a N-hydroxy succinimide, a PNP-carbonate, and a benzotrizole terminated hydrophilic polymer.

5. (Previously Amended) The method of claim 1, wherein the hydrophilic polymer and insulin protein are contacted at a molar ratio of about 10:1-1:1.

6. (Previously Amended) The method of claim 1, wherein the organic solvent is selected from the group consisting of ethanol, methanol, DMSO, dioxane, DMF, and NMP.

7. (Previously Amended) The method of claim 1, wherein the organic solvent is present at a concentration of about 0.1 to 10%.

8. (Previously Amended) The method of claim 1, wherein the insulin protein and hydrophilic polymer are contacted at a protein concentration of about 0.1 -5.0%.

9. (Previously Amended) The method of claim 1, wherein the insulin protein and hydrophilic polymer are contacted at a pH of about 5.0-7.5.

10. (Previously Amended) The method of claim 1, wherein the chelator is selected from the group consisting of polyvalent metal ion chelators, EDTA, deferoxamine (DEF), diethylenetriamine pentaacetic acid (DTPA), and bis(aminoethyl)glycolether N,N,N',N'-tetraacetic acid (EGTA).

11. (Previously Amended) The method of claim 1, wherein the chelator is present at a concentration of about 0.1-10 mM.

12. (Previously Amended) The method of claim 1, wherein the insulin protein and hydrophilic polymer are contacted at a temperature of about 4-50° C.

13. (Previously Amended) The method of claim 1, wherein the method further comprises the step of quenching formation of the conjugate prior to isolating the conjugate.

14-16. (Canceled)

17. (Previously Amended) The method of claim 1, further comprising the step of encapsulating the conjugate in a biodegradable polymer.

18. (Presently Amended) A method for preparing an insulin-PEG conjugate comprising:
(a) contacting insulin with PEG in an aqueous solution in the presence of at least one organic solvent and at least one metal chelator, ~~under conditions that~~ to form ~~promote the formation of~~ a conjugate of the insulin and PEG; and
(b) isolating the conjugate.

19. (Original) The method of claim 18, wherein the insulin comprises human insulin.

20. (Previously Amended) The method of claim 18, wherein the PEG comprises an amino-reactive PEG derivative selected from the group consisting of an aldehyde, a N-hydroxy succinimide, a PNP-carbonate, and a benzotrizole terminated hydrophilic polymer.

21-31. (Canceled)